

Claims

What is claimed is:

1. A method for determining the presence of at least one specific nucleotide sequence in a target nucleic acid reagent extracted from a biological sample, said method comprising the steps of:

(a) concurrently contacting a microarray with:

(i) a target nucleic acid reagent, said target nucleic acid reagent having a nucleotide sequence, said nucleotide sequence further including a capture sequence, and

(ii) a capture reagent, said capture reagent having at least one first arm having a label capable of emitting a detectable signal and at least one second arm having a nucleotide sequence complementary to said capture sequence of said target nucleic acid reagent;

said microarray having thereon a plurality of features, each of said plurality of features including a probe nucleotide sequence; and

(b) treating the microarray from step (a) at a temperature and for a time sufficient to induce said nucleotide sequence of said target nucleic acid to hybridize to the probe nucleotide sequence complementary thereto on the microarray, and to induce said capture reagent to hybridize to said capture sequence of said nucleotide sequence of said target nucleic acid hybridized to the microarray.

2. The method of claim 1, wherein the presence of the latter hybridization results in the emission of the detectable signal from the corresponding feature, and the absence thereof

results in no emission of the detectable signal from the corresponding feature, thus generating a detectable hybridization pattern for subsequent analysis.

3. The method of claim 1 wherein the capture reagent is selected from the group consisting of dendrimers, carbohydrates, proteins, and nucleic acids.
4. The method of claim 1 wherein the target nucleic acid reagent is cDNA.
5. The method of claim 1 wherein the capture reagent is a dendrimer.
6. The method of claim 1 wherein step (b) further comprises incubating the microarray from step (a) at a first temperature for a first period of time, and thereafter incubating the microarray from step (a) at a lower second temperature for a second period of time which may be different than the first period of time.
7. The method of claim 6 wherein said first temperature and said first period are suitable for hybridization of the target nucleic acid reagent to the probe.
8. The method of claim 6 wherein said second temperature and said second period are suitable for hybridization of the capture reagent to the target nucleic acid reagent.
9. The method of claim 6 wherein the first temperature is in the range of from about 65°C

to 75°C, and the second temperature is in the range of from about 50° to 55°C.

10. The method of claim 6 wherein the first period of time is overnight, and the second period of time is 4 to 6 hours.
11. The method of claim 1 wherein step (b) further comprises incubating the microarray from step (a) at about a temperature in the range from about 65°C to 75°C overnight, and thereafter incubating the microarray from step (a) at a temperature in the range of from about 50 to 55°C for about 4 to 6 hours.
12. The method of claim 1 further comprising forming a mixture of the at least one specific nucleotide sequence of the target nucleic acid reagent and the capture reagent and contacting the microarray with said mixture.
13. The method of claim 1 further comprising the step of utilizing a blocking nucleotide prior to step (a) to block the hybridization of said capture sequence of said target nucleic acid reagent to said capture reagent.
14. The method of claim 1 further comprising the step of pre-hybridizing a blocking nucleotide to the capture reagent prior to step (a) to prevent hybridization between said capture reagent and said capture sequence of said target nucleic acid reagent.

15. The method of claim 1 further comprising the step of pre-hybridizing a blocking nucleotide to the capture sequence of said target nucleic acid reagent prior to step (a) to prevent hybridization between said capture sequence of said target nucleic acid reagent and said capture reagent.
16. The method of claim 1 wherein step (b) further comprises incubating the microarray from step (a) at a first temperature for a first period of time, and thereafter incubating the microarray from step (a) at a higher second temperature for a second period of time which may be different than the first period of time.
17. The method of claim 13 wherein step (b) further comprises incubating the microarray from step (a) at a first temperature for a first period of time, and thereafter incubating the microarray from step (a) at a higher second temperature for a second period of time which may be different than the first period of time.
18. The method of claim 16 wherein said first temperature and said first period are suitable to allow hybridization of the target nucleic acid reagent to the probe.
19. The method of claim 16 wherein said second temperature and said second period are suitable to allow hybridization of the capture reagent to the target nucleic acid reagent.
20. The method of claim 17 wherein the first temperature is below the melt temperature of

the blocking oligonucleotide, and the second temperature is at least 5 degrees above the melt temperature of the blocking oligonucleotide yet is also a temperature suitable for binding of the capture reagent to the target nucleic acid.

21. The method of claim 17 wherein the first period of time is overnight, and the second period of time is about 3 - 5 hours.
22. The method of claim 17 wherein the first temperature is at least 5°C below the melt temperature of the blocking oligonucleotide.
23. The method of claim 12 wherein step (b) further comprises incubating the microarray from step (a) at the first temperature of about 32°C overnight, and thereafter incubating the microarray from step (a) at the second temperature of about 55°C for about 4 hours.
24. The method of claim 1 further comprising the step of utilizing a spin column to prepare said target nucleic acid reagent, prior to step (a).